

**Protocol for Submission of Laboratory Specimens for Equine Neurological Disease
Diagnosis and Surveillance
2007**

Suspected and diagnosed cases of equine viral encephalitis are (immediately) reportable to the State Veterinarian's Office (602) 542-4293 or fax (602) 542-4290. Complete the "*Equine Central Nervous System Case Tracking Form*" and fax to the Office of the State Veterinarian. Copies of *Tracking Form* are available from the State Veterinarian's Office. (If you do NOT have a fax machine, you may include the Equine CNS Case Tracking Form with the specimen submission form that accompanies the samples to the Arizona Veterinary Diagnostic Lab.)

I. Specimen collection and submission:

A. Blood for arbovirus testing:

- Red top tubes of whole blood, or separated serum (no preservatives or anticoagulants) should be refrigerated; do not freeze. Serum is used for IgM-capture ELISA testing
 - Acute Sample: within 7 days of onset of symptoms
 - If negative on first specimen, can submit a convalescent sample: 14-28 days after onset of symptoms

B. Brain for rabies and West Nile virus (WNV) testing:

- Submission of the head intact with the first two cervical vertebrae (C1 and C2) is preferable because: 1) brain and cervical spinal cord are better preserved (anatomically) when left in the skull during transport; 2) WNV virus, if present, is more likely to be detected in the anterior spinal cord; and 3) brain removal in field conditions may increase the risk of exposure to rabies.
- If sending only extracted brain, include lower brainstem, medulla and upper spinal cord.
- **The intact head should be chilled immediately after removal.** Prepare a leak proof insulated transporting container with ice or cold packs to keep the specimen chilled while in transit. The head should be double bagged.

NOTE: Rabies is on the differential diagnosis list for horses with suspected viral encephalitis. Saliva, CSF and neural tissue may be infectious, so barrier precautions are advised.

C. Submission instructions and laboratory locations:

- **Submit serum/red top tubes and/or head to the AZ Veterinary Diagnostic Lab (AzVDL) as quickly as possible.**
- AzVDL submission forms are available at:
<http://microvet.arizona.edu/AzVDL/submission.pdf>
- If you do not have an AzVDL specimen submission form, include the following information:
 - **Name of submitting veterinarian & veterinary clinic**
 - **Phone number, fax number, and address of veterinarian for results**
 - **Name, Address, phone number of owner**
 - **Physical address where horse resides** (Physical address of where horse lives is needed by the county health department for West Nile virus surveillance and response purposes).
 - **Name, age and breed of horse**
 - **Date of onset of clinical signs**
 - **Date of sample collection**
 - **Date of euthanasia or death, if applicable**
 - **Brief summary of neurologic illness**
 - **Number of other animals on farm/ranch**
 - **Number of affected animals on farm/ranch**
- The submittal information for each specimen should be placed in a leak proof plastic bag and attached to the corresponding container.
- Include the *Equine CNS Case Tracking Form* if you are not able to fax it to the State Veterinarian's Office.
- Samples should be shipped so as NOT to arrive on the weekend at the AzVDL. In the event that rabies is a more likely differential diagnosis (e.g. due to origin of horse, history of previous contact with potentially rabid animal, or other), rabies may need to be quickly ruled in or out. Consult with AZ Department of Health Services Vector-Borne & Zoonotic Disease Section staff at (602) 364-3852 to determine if the sample should be shipped to the Arizona State Health Lab rather than AzVDL.

D. Laboratories

- AzVDL, 2831 N. Freeway, Tucson 85705, Phone: (520) 621-2356
- AZ State Health Lab, 250 N. 17th Ave, Phoenix, AZ 85007 Phone: (602) 542-1190
- Regional State Health Lab, 416 West Congress St., Tucson, AZ 85701, (520) 628-6360
- AZ State Health Lab in Flagstaff (no on-site testing; can arrange shipment) (928) 226-1154

There is no charge for rabies testing. At this time, there is no charge for WNV IgM-capture ELISA on serum samples, or immunohistochemistry (IHC) on tissue samples thanks to funding from CDC and the Arizona Department of Health Services. A fee is charged for more extensive diagnostic testing (culture, toxicology, etc.). Call the AzVDL for specifics.

II. **Case Definition of West Nile Virus Infection for Arizona Horses**

Because the presence of WNV was established in Arizona in 2003, the WNV case definition for horses is:

1. compatible clinical signs and positive IgM-capture ELISA; or
2. compatible clinical signs and the demonstration of WNV DNA through either
 - Polymerase Chain Reaction (PCR) testing of tissue samples; or
 - Immunohistochemistry testing of tissues

Equine Central Nervous System Case Tracking Form

Veterinarians: Report suspected cases within 4 hours & fax to State Vet's office (602) 542-4290

(No fax machine? Send with samples to Arizona Veterinary Diagnostic Laboratory)

Please complete at least the bolded & underlined boxes; it is crucial information for WNV surveillance

Horse Name		<u>Date Form Completed:</u>		Completed by:																									
Owner Name (Last) (First)		Horse's Age	Gender <input type="checkbox"/> Mare <input type="checkbox"/> Gelding <input type="checkbox"/> Stallion	Breed <input type="checkbox"/> (1) Quarter Horse <input type="checkbox"/> (2) Thoroughbred <input type="checkbox"/> (3) Arabian <input type="checkbox"/> (4) Draft <input type="checkbox"/> (5) Pony <input type="checkbox"/> (6) Miniature <input type="checkbox"/> (7) Donkey <input type="checkbox"/> (8) Mule <input type="checkbox"/> (9) Other _____	<u>Outcome</u> <input type="checkbox"/> Euthanized <input type="checkbox"/> Died <input type="checkbox"/> Survived <input type="checkbox"/> Alive @ time of collection but Unknown outcome																								
<u>Street Address (where horse resides)</u>		Owner Telephone # ()		Pregnant <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown																									
<u>Closest cross streets</u>		<u>County or Tribal Residence</u>																											
<u>City</u>		State AZ	<u>Zip code</u>	GPS Coordinates																									
<u>Diagnosis or suspected reportable condition</u>																													
<u>Date of onset</u>		<input type="checkbox"/> Arizona VDLab <input type="checkbox"/> New Mexico VDLab <input type="checkbox"/> Other lab _____ <u>Accession no.</u> (if known):																											
<u>Attending Veterinarian</u>		<u>Veterinary Clinic Name</u>		<u>Telephone #</u> <u>Veterinarian's Fax #</u>																									
<u>City</u>		State (please list of other than AZ) AZ																											
<u>Risk Factor Assessment:</u> In-state travel? <input type="checkbox"/> Yes <input type="checkbox"/> No Date ____/____/____ Location _____ Out-of-state travel? <input type="checkbox"/> Yes <input type="checkbox"/> No Date ____/____/____ Location _____		<u>Clinical Signs:</u> <input type="checkbox"/> Weakness <input type="checkbox"/> Ataxia <input type="checkbox"/> Abnormal mentation <input type="checkbox"/> Fever Max temp _____ <input type="checkbox"/> Fasciculation <input type="checkbox"/> Anorexia <input type="checkbox"/> Cranial Nerve Deficits <input type="checkbox"/> Flaccid paralysis <input type="checkbox"/> Teeth Grinding <input type="checkbox"/> Unable to rise <input type="checkbox"/> Flaccid paralysis <input type="checkbox"/> Other – <i>specify</i> _____ _____ _____ _____		For Use by State Veterinarian Office, Arizona Dept. of Health Services, or county health department Laboratory Results: <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="padding: 5px;">Rabies Positive / Negative (circle one)</td> <td style="padding: 5px;">WNV IgM ELISA (mark below)</td> <td style="padding: 5px;"></td> </tr> <tr> <td style="padding: 5px;">IHC for WNV</td> <td colspan="2" style="padding: 5px;">PCR for WNV</td> </tr> </table>		Rabies Positive / Negative (circle one)	WNV IgM ELISA (mark below)		IHC for WNV	PCR for WNV																			
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<u>Vaccination status:</u> WNV <input type="checkbox"/> Yes <input type="checkbox"/> No Date ____/____/____ WEE <input type="checkbox"/> Yes <input type="checkbox"/> No Date ____/____/____ EEE <input type="checkbox"/> Yes <input type="checkbox"/> No Date ____/____/____ VEE <input type="checkbox"/> Yes <input type="checkbox"/> No Date ____/____/____ Rabies <input type="checkbox"/> Yes <input type="checkbox"/> No Date ____/____/____		For use by State Veterinarian Office, Arizona Dept. of Health Services, or county health department <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="padding: 5px;"><input type="checkbox"/> Confirmed case</td> <td style="padding: 5px;">Entered into:</td> </tr> <tr> <td style="padding: 5px;"><input type="checkbox"/> Probable case</td> <td style="padding: 5px;"><input type="checkbox"/> ADHS Database</td> </tr> <tr> <td style="padding: 5px;"><input type="checkbox"/> Suspect case</td> <td style="padding: 5px;"><input type="checkbox"/> SVO Database</td> </tr> <tr> <td colspan="2" style="padding: 5px;"><input type="checkbox"/> Ruled out/Non-case</td> </tr> </table>				<input type="checkbox"/> Confirmed case	Entered into:	<input type="checkbox"/> Probable case	<input type="checkbox"/> ADHS Database	<input type="checkbox"/> Suspect case	<input type="checkbox"/> SVO Database	<input type="checkbox"/> Ruled out/Non-case																	
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Other horses on farm showing signs of WNV? <input type="checkbox"/> Yes <input type="checkbox"/> No # of horses ill _____		<input type="checkbox"/> reported to county health department <input type="checkbox"/> reported to state health department <input type="checkbox"/> reported to Office of State Veterinarian																											
<u>Specimen Type Submitted to Lab and Date (s) Collected:</u> <input type="checkbox"/> Serum (Acute) ____/____/____ <input type="checkbox"/> Serum (Convalescent) ____/____/____ <input type="checkbox"/> CSF ____/____/____ <input type="checkbox"/> Brain / CNS Tissue ____/____/____ <input type="checkbox"/> Other ____/____/____		Serology Results <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="padding: 5px;"></th> <th style="padding: 5px;">Acute</th> <th style="padding: 5px;">Convalescent</th> </tr> <tr> <td style="padding: 5px;"><input type="checkbox"/> East Equine Encephalitis</td> <td style="padding: 5px;">_____</td> <td style="padding: 5px;">_____</td> </tr> <tr> <td style="padding: 5px;"><input type="checkbox"/> West Equine Encephalitis</td> <td style="padding: 5px;">_____</td> <td style="padding: 5px;">_____</td> </tr> <tr> <td style="padding: 5px;"><input type="checkbox"/> St. Louis Encephalitis</td> <td style="padding: 5px;">_____</td> <td style="padding: 5px;">_____</td> </tr> <tr> <td style="padding: 5px;"><input type="checkbox"/> Venezuelan Encephalitis</td> <td style="padding: 5px;">_____</td> <td style="padding: 5px;">_____</td> </tr> <tr> <td style="padding: 5px;"><input type="checkbox"/> West Nile Virus</td> <td style="padding: 5px;">_____</td> <td style="padding: 5px;">_____</td> </tr> <tr> <td style="padding: 5px;"><input type="checkbox"/> Equine Herpes Virus</td> <td style="padding: 5px;">_____</td> <td style="padding: 5px;">_____</td> </tr> <tr> <td style="padding: 5px;"><input type="checkbox"/> Other _____</td> <td style="padding: 5px;">_____</td> <td style="padding: 5px;">_____</td> </tr> </table>					Acute	Convalescent	<input type="checkbox"/> East Equine Encephalitis	_____	_____	<input type="checkbox"/> West Equine Encephalitis	_____	_____	<input type="checkbox"/> St. Louis Encephalitis	_____	_____	<input type="checkbox"/> Venezuelan Encephalitis	_____	_____	<input type="checkbox"/> West Nile Virus	_____	_____	<input type="checkbox"/> Equine Herpes Virus	_____	_____	<input type="checkbox"/> Other _____	_____	_____
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